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3-Deoxyglucosone and AGEs in uremic complications: Inactivation of glutathione peroxidase by 3-deoxyglucosone

Toshimitsu Niwa and Saori Tsukushi

Nagoya University Daiko Medical Center, Nagoya, Japan

3-Deoxyglucosone and AGEs in uremic complications: Inactivation of glutathione peroxidase by 3-deoxyglucosone. 3-Deoxyglucosone (3-DG) is accumulated not only in uremic serum but also in uremic erythrocytes. 3-DG rapidly reacts with protein amino groups to form advanced glycation end products (AGEs) such as imidazolone, pyrraline, and N^{ϵ}-(carboxymethyl)lysine, among which imidazolone is the AGE that is most specific for 3-DG. In diabetes, hyperglycemia enhances the synthesis of 3-DG via the Maillard reaction and the polyol pathway and thereby leads to its high plasma and erythrocyte levels. In uremia, however, the decreased catabolism of 3-DG that may be due to the loss of 3-DG reductase activity in the end-stage kidneys may lead to a high plasma 3-DG level. The elevated 3-DG levels in uremic patients may promote the formation of AGEs such as imidazolone in erythrocytes, aortas, and dialysis-related amyloid deposits. Treatment with an aldose reductase inhibitor reduced the erythrocyte levels of 3-DG and AGEs such as imidazolone in diabetic uremic patients. This finding demonstrates an important role of the polyol pathway in the formation of erythrocyte 3-DG and AGEs. The erythrocyte levels of 3-DG are elevated in not only diabetic uremic but also nondiabetic uremic patients. 3-DG showed some cytotoxicities by inducing intracellular oxidative stress. In contrast, oxidative stress was demonstrated to cause accumulation of intracellular 3-DG. Recently, we have demonstrated that 3-DG inactivates intracellular enzymes such as glutathione peroxidase, a key enzyme in the detoxification of hydrogen peroxide. Thus, intracellular accumulation of 3-DG may enhance oxidative stress by inactivating the antioxidant enzymes. In conclusion, 3-DG may play a principal role in the development of uremic complications, such as dialysis-related amyloidosis, atherosclerosis, and enhanced oxidative stress.

Glucose reacts nonenzymatically with protein amino groups to initiate glycation, the early stage of the Maillard reaction. This process begins with the conversion of reversible Schiff base adducts to stable, covalently bound Amadori rearrangement products. The levels of the Amadori products on numerous proteins are elevated in proportion to the degree of hyperglycemia in diabetes mellitus. In the intermediate stage of the Maillard reaction, the Amadori products can then undergo multiple dehydration and rearrangements to produce highly reactive carbonyl compounds such as 3-deoxyglucosone (3-DG), which reacts again with free amino groups, leading to cross-linking and browning of the proteins via the formation of advanced glycation end products (AGEs) in the late stage of the Maillard reaction.

Several compounds such as N^{ϵ}-(carboxymethyl)lysine (CML), pyrraline, pentosidine, crosslines, and imidazolone have been proposed as candidates for the structures of AGEs. CML is an AGE formed on protein by combined nonenzymatic glycation and oxidation (glycoxidation) reactions, but is also formed during metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of protein. Thus, both glycoxidation and lipid peroxidation may be important sources of CML in tissue proteins in vivo, and CML may be a general marker of oxidative stress and long-term damage to protein in aging, atherosclerosis, diabetes, and dialysis-related amyloidosis. Pentosidine is also produced as a glycoxidation product. Although these AGEs and glycoxidation products are implicated in the development of diabetic complications, these compounds are present at only trace concentrations in tissue proteins and account for only a fraction of the chemical modifications in AGE proteins prepared in vitro. Pyrraline is an AGE formed by a nonenzymatic reaction initiated by glucose with lysine residues on proteins, and this reaction involves 3-DG as an intermediate.

Imidazolone was isolated as a novel AGE in vitro from the incubation mixture of 3-DG and an arginine derivative. The formation of imidazolone by incubating 3-DG with arginine is very rapid, reaching a maximum concentration within 24 hours [1]. Incubation of 3-DG with proteins also leads to the formation of pyrraline [2], pentosidine [3], CML [4, 5], as well as imidazolones [6, 7]. Among these AGEs, imidazolone is the AGE that is most specific for the in vivo involvement of 3-DG in the modification of tissue proteins. We first detected imidazolone in vivo in β_2-microglobulin amyloid deposits obtained from hemodialysis (HD) patients [6] and also in the kidneys and aortas of diabetic patients [7] by

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immunohistochemistry using a monoclonal anti-imidazolone antibody (AG-1).

Polyol pathway

The polyol pathway may be involved in the formation of 3-DG. 3-DG was demonstrated to be nonenzymatically formed in the absence of amino groups from fructose, an oxidized product of sorbitol by sorbitol dehydrogenase in the polyol pathway [8]. 3-DG has also been reported to be a biophysical hydrolysis product of fructose 3-phosphate, which was identified in the lens and hearts of diabetic rats [9, 10]. Fructose 3-phosphate is considered to be enzymatically produced from fructose [10, 11]. These data suggest that the formation of 3-DG may occur via the polyol pathway in vivo and may be involved in the development of diabetic and uremic complications.

TOXICITY OF 3-DEOXYGLUCOSONE

Induction of heparin-binding epidermal growth factor-like growth factor

3-Deoxyglucosone selectively induced heparin-binding epidermal growth factor (HB-EGF)-like growth factor mRNA in rat aortic smooth muscle cells and increased the secretion of HB-EGF from rat aortic smooth muscle cells [12]. 3-DG augmented intracellular peroxides prior to the induction of HB-EGF mRNA. *N*-acetyl-L-cysteine and aminoguanidine suppressed 3-DG–induced HB-EGF mRNA and intracellular peroxide levels in rat aortic smooth muscle cells. 3-DG induces HB-EGF by increasing the intracellular peroxide levels. Since HB-EGF is known as a potent mitogen for smooth muscle cells and is abundant in atherosclerotic plaques, the induction of HB-EGF by 3-DG with the simultaneous elevation of intracellular peroxides may trigger atherogenesis in uremic patients.

Induction of apoptosis

3-Deoxyglucosone induced apoptosis of monocytic leukemia cells at physiological concentrations [13]. Both ladder formation of DNA and nuclear fragmentation were observed in the 3-DG–treated cells, indicating that apoptotic cell death was induced. The fluorescent intensity of an oxidant-sensitive dye was increased in the cells. Apoptosis and intracellular oxidant levels were enhanced by an inhibitor of glutathione biosynthesis and were partially blocked by *N*-acetyl-L-cysteine, an antioxidant. Thus, intracellular oxidative stress is a cause of the apoptosis induced by 3-DG.

The gene of aldehyde reductase, which catalyzes the reduction of 3-DG, was overexpressed in rat pheochromocytoma PC12 cells [14]. Cytotoxicity of 3-DG to induce apoptotic cell death was determined by fluorescent microscopy. In the aldehyde reductase gene-transfected cells, the cytotoxicity of 3-DG and apoptotic cell death were decreased. Thus, the intracellular aldehyde reductase protects neural cells from the cytotoxicity of 3-DG, and thus, neural cells, which normally express a low level of aldehyde reductase, might be susceptible to diabetic complications caused by 3-DG.

Neurotoxicity on cultured cortical neurons

3-Deoxyglucosone showed neurotoxicity on cultured cortical neurons, and the effects were associated with reactive oxygen species production followed by neuronal apoptosis [15]. Pretreatment with *N*-acetylcysteine induced neuroprotection against 3-DG. Cotreatment, but not pretreatment, with aminoguanidine protected neurons against the neurotoxicities of both compounds.

Teratogenicity on cultured whole embryo

Rat embryos exposed to high glucose levels in vitro showed severe dysmorphogenesis and a 17-fold increased concentration of 3-DG compared with control embryos cultured in a low glucose concentration. Exogenous 3-DG added to the medium of control cultures yielded an increased embryonic malformation rate and a 3-DG concentration similar to that of embryos cultured in high glucose [16]. Thus, 3-DG is a teratogenic factor. The antiteratogenic effect of SOD administration appears to occur downstream of 3-DG formation, suggesting that 3-DG accumulation leads to superoxide-mediated embryopathy.

Suppression of cell proliferation

3-Deoxyglucosone suppressed the proliferation of various cell lines by inhibition of DNA synthesis. The cells proliferating actively, in which the intracellular glutathione sulfhydryl (GSH) concentration have been reported to be lower, were more susceptible to the inhibitory effects of 3-DG on the cell-cycle progression during the S phase [17].

Inactivation of glutathione reductase

3-Deoxyglucosone, and the other reactive endogenous aldehydes such as methylglyoxal, and xylosone inactivated glutathione reductase, a central antioxidant enzyme [18]. However, these 2-oxoaldehydes were much less effective than 4-hydroxynonenal, which is a product of oxidative degradation of unsaturated lipids and is an endogenous reactive α,β-unsaturated aldehyde with numerous biological activities. In addition to 4-hydroxynonenal, 3-DG may stimulate a positive feedback loop by inactivating glutathione reductase that enhances the potential for oxidative damage.

Inactivation of glutathione peroxidase

We have demonstrated that 3-DG inactivated glutathione peroxidase, a key antioxidant enzyme to detoxify

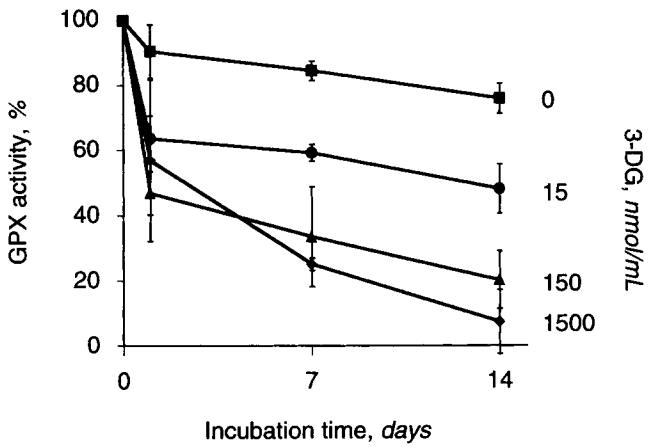

Fig. 1. Time course of glutathione peroxidase (GPX) activity by incubating with 3-deoxyglucosone (3-DG). GPX from human erythrocytes (Sigma Chemical, St. Louis, MO, USA) was incubated with 3-DG at (■) 0, (●) 15, (▲) 150, or (◆) 1500 nmol/mL in 100 mmol/L Tris-HCl buffer, pH 8.0, containing 0.5 mmol/L EDTA. 3-DG inactivated GPX time- and dose-dependently, even at a concentration of 15 nmol/mL, which is comparable to that in erythrocytes from nondiabetic HD patients (15.4 ± 1.8 nmol/mL, mean ± SE, $N = 12$). GPX activity was measured using the coupled enzymatic assay. Briefly, a sample solution (10 μL) or water (10 μL) as a control was preincubated with 100 μL of 1 mol/L Tris-HCl (pH 8.0) containing 5 mmol/L EDTA, 20 mL of 100 mmol/L GSH in TE buffer (×10 dilution), 100 μL of 10 U/mL glutathione reductase in TE buffer (×10 dilution), 100 μL of 2 mmol/L NADPH in TE buffer (×10 dilution), and 660 μL of water at 37°C for five minutes. Then the mixture was incubated with 10 μL of 7 mmol/L t-butyl hydroperoxide at 37°C for five minutes, and absorbance at 340 nm was measured. GPX activity was calculated as μmol NADPH/min/L. GPX activity at 0 day (incubation time) was approximately 350 μmol NADPH/min/L and was expressed as 100% in the figure.

hydrogen peroxide (Fig. 1). 3-DG inactivated glutathione peroxidase time- and dose-dependently, even at a concentration of 15 nmol/mL, which is comparable to that in erythrocytes from nondiabetic HD patients. This inactivation may be due to the modifications of the enzyme with AGEs such as imidazolone.

Suppression of enzyme activities of glucose metabolism

3-Deoxyglucosone inhibited the activities of mouse hepatic enzymes responsible for glucose metabolism [19]. 3-DG markedly inhibited hexokinase and glucose-6-phosphate dehydrogenase activities, while it scarcely affected glucokinase, glucose-6-phosphatase, and phosphofructokinase activities. Thus, 3-DG inhibits the intake of glucose in the liver and may be involved in the aggravation of hyperglycemia in diabetes.

IMPLICATIONS OF 3-DG AND AGES IN UREMIA

Serum 3-DG levels are elevated not only in diabetic patients [20] but also in uremic patients [5, 21]. Serum levels of 3-DG are increased in HD, continuous ambulatory peritoneal dialysis (CAPD), and undialyzed uremic patients as compared with normal subjects and diabetic patients [5]. The serum levels of 3-DG decrease after HD with a mean reduction rate of 67%, because 3-DG is a small molecule with the molecular weight of 162. Even after HD, however, the serum levels of 3-DG are significantly higher than those in normal subjects. The serum levels of 3-DG in the undialyzed uremic, HD, and CAPD patients are high even as compared with the diabetic patients. In undialyzed uremic patients, serum 3-DG is positively correlated with serum creatinine.

A major urinary metabolite of 3-DG administered to rats was identified as 3-deoxyfructose [22]. 3-Deoxyfructose has also been detected in human urine and plasma [23]. Thus, 3-DG is detoxified by the reducing enzyme to 3-deoxyfructose, which is then excreted to urine. The elevated serum levels of 3-DG in diabetic patients may be due to the increased production of 3-DG via the Maillard reaction resulting from hyperglycemia. However, the reason that the serum 3-DG level in uremic patients is increased is not clear at present. Because 3-DG is not excreted into urine in healthy subjects [23], the reduced renal clearance of 3-DG by the diseased kidneys cannot explain the elevated serum 3-DG level in uremic patients. Nicotinamide adenine dinucleotide phosphate (NADPH)-dependent 3-DG reducing enzyme activity was detected in the extracts of various monkey tissues, among which kidney exhibited the highest specific activity [24]. The activity of 3-DG–reducing enzymes, which convert 3-DG to 3-deoxyfructose, may be reduced in uremia, leading to the accumulation of 3-DG in uremic serum.

Two competitive enzyme-linked immunosorbent assays (ELISAs) were developed for measurement of serum AGEs, using a monoclonal antibody directed against imidazolone, and a polyclonal antibody directed against keyhole limpet hemocyanin-AGE [25]. Each of the assays showed significant elevation of serum AGE levels in HD patients compared with healthy subjects. These methods will be particularly valuable for monitoring the removal of AGEs by novel dialysis membranes, as well as the effect of new drugs for the inhibition of their formation.

β_2-Microglobulin isolated from the amyloid deposits in patients with dialysis-related amyloidosis has been demonstrated to be modified with AGEs [6, 26–28]. Furthermore, we demonstrated that imidazolone and CML were localized to amyloid deposits in patients with dialysis-related amyloidosis by immunohistochemistry using monoclonal anti-imidazolone and anti-CML antibodies [4, 27, 28]. Incubation of β_2-microglobulin with 3-DG under physiological conditions emitted fluorescence characteristic for AGE (excitation, 370 nm; emission, 440 nm) and caused AGE modification and dimer formation of β_2-microglobulin, as demonstrated by Western blotting using the same monoclonal antibodies. The AGE-modified dimer of β_2-microglobulin could be extracted from the amyloid tissue of a patient with dialysis-related amy-

loidosis. 3-DG showed more intense and faster reactivity with β_2-microglobulin to form AGEs (imidazolone and CML) and dimer as compared with glucose, and aminoguanidine suppressed the AGE and dimer formation of β_2-microglobulin by 3-DG.

3-Deoxyglucosone accumulating in uremic serum promotes the modification of β_2-microglobulin with AGEs, including imidazolone and CML mainly after deposition of β_2-microglobulin as amyloid [28]. Once amyloid is formed, it is difficult to degrade. Then it may react with 3-DG and the other precursors of AGEs and become modified with imidazolone and CML even in normoglycemia after a long-term period of deposition as β_2-microglobulin amyloid. Furthermore, a concomitant generation of oxygen radicals, which occurs in HD patients, may enhance the aggregation of amyloid β_2-microglobulin [29, 30]. Thus, a uremic state in which 3-DG is accumulated [5, 21] and generation of oxygen radicals is increased accelerates the AGE modification and aggregation of β_2-microglobulin amyloid.

We demonstrated that erythrocyte 3-DG is produced via the polyol pathway in diabetic HD patients [31]. The erythrocyte levels of 3-DG were measured in the patients before and after an aldose reductase inhibitor (epalrestat) treatment. The erythrocyte levels of sorbitol, 3-DG, and AGEs such as imidazolone and CML were significantly elevated in diabetic HD patients as compared with healthy subjects. The administration of the aldose reductase inhibitor significantly decreased the erythrocyte levels of sorbitol, 3-DG, and imidazolone and tended to decrease the CML level. Thus, AGEs as well as 3-DG in the erythrocytes of these patients are produced mainly via the polyol pathway. The aldose reductase inhibitor may prevent diabetic and uremic complications associated with AGEs.

Oxidative stress was demonstrated to cause intracellular accumulation of 3-DG. Oxidative stress induced by toxic concentration of hydrogen peroxide and 1-chloro-2,4-dinitrobenzene (CDNB) in murine P388D1 macrophages caused a marked increase in cytosolic 3-DG during necrotic cell death [32]. 3-DG is enzymatically detoxified in cells by NADPH-dependent reductase and $NAD(P)^+$-dependent dehydrogenase. The accumulation of 3-DG in toxicant-treated cells may be due to the decreased availability of pyridine nucleotide cofactors for the detoxification of 3-DG. Thus, impairment of 3-DG detoxification is cytotoxic, and this may contribute to toxicity associated with GSH oxidation and S conjugation in oxidative stress and chemical toxicity, and to the chronic pathogenesis associated with uremia as well as diabetes mellitus, where there is oxidative stress and the formation of 3-DG is increased.

We have demonstrated that erythrocyte levels of 3-DG are elevated in diabetic HD patients [31] and nondiabetic HD patients [33]. 3-DG inactivates intracellular antioxidant enzymes such as glutathione peroxidase and glutathione reductase. The increased erythrocyte levels of 3-DG in HD patients may cause inactivation of the antioxidant enzymes in erythrocytes. Thus, intracellular accumulation of 3-DG may further enhance the oxidative stress in HD patients.

CONCLUSION

3-Deoxyglucosone was originally detected as a reactive intermediate of the Maillard reaction in vitro. However, recent studies have demonstrated that 3-DG is synthesized in human bodies via the Maillard reaction and the polyol pathway. The serum and erythrocyte levels of 3-DG are increased in uremic patients. 3-DG rapidly reacts with protein amino groups to form AGEs such as imidazolone. The involvement of 3-DG in modification of tissue proteins has been demonstrated in uremic patients by immunohistochemistry and immunochemistry using a monoclonal anti-imidazolone antibody. Although the chemical AGE structures responsible for biological activities of AGE-modified proteins are not yet known, 3-DG has some toxic effects on cells and enzymes. 3-DG shows cytotoxicity by inducing intracellular oxidant stress. In contrast, oxidant stress was demonstrated to cause accumulation of intracellular 3-DG. The intracellularly accumulated 3-DG inactivates antioxidant enzymes such as glutathione peroxidase, thereby enhancing the oxidative stress. These studies have emphasized an important role of 3-DG and AGEs in the development of uremic complications.

Reprint requests to Dr. Toshimitsu Niwa, Nagoya University Daiko Medical Center, 1-1-20, Daiko-minami, Higashi-ku, Nagoya 461-0047, Japan.
E-mail: tniwa@med.nagoya-u.ac.jp

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